Full-length article

# G protein-coupled inwardly rectifying potassium channels in dorsal root ganglion neurons[1]

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## Key words

## Abstract

**Aim:** G protein-coupled inwardly rectifying potassium channels (GIRK) are important for neuronal signaling and membrane excitability. In the present study, we intend to find whether GIRK channels express functionally in adult rat dorsal root ganglion (DRG) neurons. **Methods**: We used RT-PCR to detect mRNA for 4 subunits of GIRK in the adult DRG. The whole-cell patch clamp recording was used to confirm GIRK channels functionally expressed. **Results**: The mRNA for the 4 subunits of GIRK were detected in the adult DRG. $GTP_\gamma S$ enhanced inwardly rectifying potassium ($K^+$) currents of the DRG neurons, while $Ba^{2+}$ inhibited such currents. Furthermore, the GIRK channels were shown to be coupled to the $GABA_B$ receptor, a member of the G protein-coupled receptor family, as baclofen increased the inwardly rectifying $K^+$ currents. **Conclusion:** GIRK channels are expressed and functionally coupled with $GABA_B$ receptors in adult rat DRG neurons.

## Introduction

The G protein-coupled inwardly rectifying potassium channel (GIRK) is a member of the inwardly rectifying potassium ($K^+$) channel family. Five GIRK subunits have been identified in mammals and *Xenopus oocytes*, named GIRK1–5 (also designated Kir3.1–5)[1]. GIRK channels are homotetramers or heterotetramers. *In vivo*, GIRK1 has to assemble with the other GIRK family members to form functional channels[1]. GIRK channels are downstream effectors of G protein-coupled receptors, and they can be activated by direct binding of the $\beta\gamma$ subunits of G protein to the channels[2–4]. A large number of neuromodulators are capable of opening GIRK channels in a G protein-dependent, membrane-delimited fashion, including adenosine, serotonin, dopamine, adrenalin, acetylcholine, opioids, and somatostatin[5–7]. GIRK channels are distributed in the pancreas, heart and brain, and play pivotal roles in controlling insulin release, vascular tone, heart rate, neuronal signaling, and membrane excitabil-

ity[8]. For example, the activation of the heart muscarinic $m_2$ receptor slows the heart rate by activating GIRK1/GIRK4 heterotetrameric channels[9,10]. In the hippocampus, baclofen can activate GIRK channels, which produce a reversible reduction of EPSP[11].

Dorsal root ganglion (DRG) neurons are the primary neurons for somatic and visceral afferentation. Their bodies reside in the DRG, and extend axons that innervate both peripheral and central targets. A diverse group of ligand- and voltage-gated ion channels transduce innocuous and noxious (nociceptive) stimuli into depolarizations that are conducted along axons and finally converted into neurotransmitter release[12]. There are many G protein-coupled receptors on DRG neurons, such as the $\mu$ opioid receptor[13], the $GABA_B$ receptor[14], the oxytocin receptor[15], SNSR4/mrgX1[16], *etc*. The activation of these receptors can affect the conductance of DRG neurons. For example, the activation of human sensory neuron-specific G protein-coupled receptors (SNSR), expressed solely in small diameter primary sensory

neurons, modulates neuronal $K^+$ channels and synaptic transmission[16]; clonidine, an $\alpha_2$-adrenoceptor agonist, inhibits hyperpolarization-activated currents in rat DRG neurons[17].

Although GIRK channels are very important to mediate signaling through G protein-coupled receptors, no data concerning the expression of functional GIRK channels in adult DRG neurons has been reported. In the present report, we show that 4 subtypes of GIRK channel subunits are expressed on DRG neurons, and the GIRK channels are functionally coupled to the $GABA_B$ receptor.

## Materials and methods

**RT-PCR analysis** The total RNA from these DRG was extracted using the RNA-Clean kit (TaKaRa, Dalian, China) and treated with DNase I (RNase-free from TaKaRa, Dalian, China). cDNA were synthesized using the M-MLV RTase cDNA Synthesis Kit (TaKaRa, China). PCR was performed using the following primer pairs: GIRK1, forward 5'-CGGCA-GCGGTTCGTGGACAAG-3' and reverse 5'-TGGCTGGTGC-TATTAAAGGGGAAGACAT-3'; GIRK2, forward 5'-AGCC-GAGACAGGACCAAAAGGAAAATC-3' and reverse 5'-ACGGGGTGCTGGTCTCATAGGTCTC-3'; GIRK3, forward 5'-CTACCGCTACCTGACCGACCTGTT-3' and reverse 5'-AGCCCCTTCTTCCTCCACCTTCT-3'; GIRK4, forward 5'-GGAGAAGACCGGCAAGTGTAACG-3' and reverse 5'-GCCCCCAAGCAAAGGAGGAC-3'. For GIRK1, PCR amplification was performed for 35 cycles using Taq polymerase (TaKaRa, China), each cycle consisted of 30 s at 94 °C, 30 s at 60 °C, and 1 min at 72 °C. For GIRK2, PCR amplification was performed for 35 cycles using LA *Taq* polymerase and buffer I (TaKaRa, China), each cycle consisted of 40 s at 94 °C, 40s at 60 °C, and 1 min at 72 °C. For GIRK3, PCR amplification was performed for 35 cycles using LA *Taq* polymerase and buffer II (TaKaRa, China), each cycle consisted of 40 s at 94 °C, 40 s at 60 °C, and 1 min at 72 °C. For GIRK4, PCR amplification was performed for 35 cycles using Taq polymerase (TaKaRa, China), each cycle consisted of 40 s at 94 °C, 40 s at 58 °C, and 1 min at 72 °C. The amplified samples were analyzed by standard agarose gel electrophoresis. DL-2000 DNA marker (TaKaRa, China) or BenchTop 1 kb DNA ladder (Promega, Madison, Wisconsin, USA) was used. All the products were further confirmed by DNA sequencing.

**Preparation of isolated DRG** The animal experiments were carried out in adherence with the National Institutes of Health Guidelines on the Use of Laboratory Animals and were approved by Second Military Medical University Committee on Animal Care. Adult male Sprague-Dawley rats (150–200 g), from the Experimental Animal Center in Sec-

ond Military Medical University, were housed with access to food and water ad libitum under standard conditions in a 12/12 light dark cycle. The adult rats were deeply anaesthetized with 40 mg/mL sodium pentobarbital and killed by decapitation. The DRG were removed and digested in solution containing 1 mg/mL collagenase type 1A (Sigma, St Louis, MO, USA), 0.4 mg/mL trypsin type I (Sigma, USA), and 0.1 mg/mL Dnase I (Sigma, USA) in DMEM at 37 °C for 40 min, triturated, and plated on coverslips. The cells were observed with an Olympus IX71 inverted microscope (Olympus Optical, Tokyo, Japan).

**Whole cell patch-clamp recording** The cells were bathed in normal Ringer's solution containing (in mmol/L): NaCl, 140; KCl, 5; $CaCl_2$, 2; $MgCl_2$, 1; glucose, 10 and HEPES, 10; pH 7.4 adjusted with NaOH. When an increase in $[K^+]_o$ was needed, NaCl was reduced by equal amounts to keep the osmolarity unchanged. Patch pipettes had a resistance of 6–8 MΩ. The pipette solution consisted of (in mmol/L): KCl, 120; NaCl, 20; HEPES, 10; glucose·$H_2O$, 10; EGTA, 1; $Na_2ATP·3H_2O$, 3; $MgCl_2·6H_2O$, 3; NaGTP, 0.3; pH 7.2 adjusted with NaOH. Recordings were made with an Axon-patch 200B amplifier and pClampex 9.0 software (Axon Instruments, Sunnyvale, CA, USA). Analogue signals were filtered at 2 kHz, sampled at 10 kHz and stored on a PC hard disk for further analysis. Baclofen and $GTP_\gamma S$ used in the experiments were from Sigma. Data analysis was performed using Clampfit 9.0 (Axon Instruments, USA) and Origin 7.0 (OriginLab Corporation, Northampton, MA, USA). Data were all presented as mean±SD.

## Results

**GIRK channel subunits in rat DRG** In the RT-PCR analysis of total RNA extracted from DRG, the oligonucleotide primers for GIRK1−4 showed amplification of products of expected sizes (Figure 1). These results demonstrated that the mRNA for the 4 subunits of GIRK were expressed in the adult DRG.

**GIRK currents in DRG neurons** To determine whether the GIRK subunits in the DRG neurons contributed to form functional channels, voltage-dependent whole-cell currents from isolated DRG neurons were recorded and characterized. After dissociation procedures, DRG neurons dendrites might have been destroyed, and only neurons with a thin axon were selected to be tested. Neurons retained their active properties after isolation as demonstrated by the voltage-clamp protocol. Successive depolarizing voltage steps evoked typical sequences of fast inward (presumed $Na^+$) currents followed by slow outward (presumed $K^+$) currents (Figure 2).
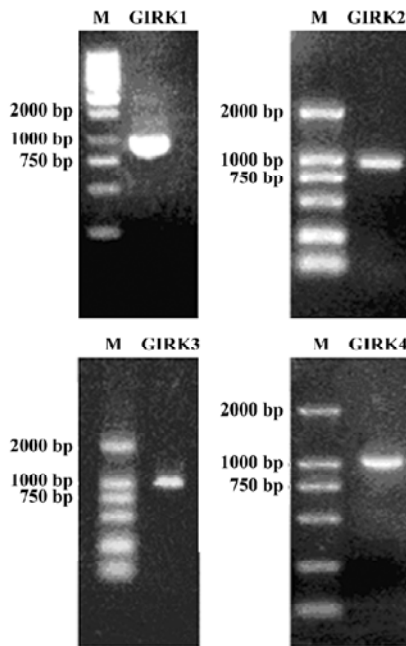
**Figure 1.** mRNA for GIRK1–4 channel subunits were expressed in DRG of adult rats. GIRK-specific oligonucleotide pairs were used to determine the expression of mRNA for the GIRK channel subunits in the total RNA extracted from DRG. M, DNA marker. Numbers on the left side indicate the positions of molecular weight markers in base pairs (bp). The RT-PCR product for GIRK1 is expected 1000 bp; GIRK2, 958 bp; GIRK3, 957 bp; GIRK4, 1003 bp. All the products were further confirmed by DNA sequencing.
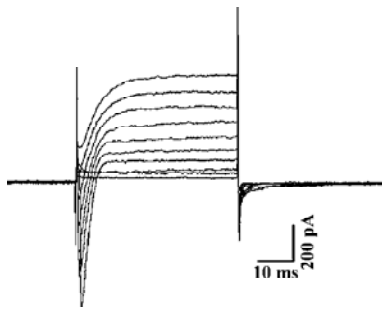


**Figure 2.** Electrical properties of acutely isolated DRG neurons. Inward and subsequent outward currents responded in a voltage-clamped DRG neuron, evoked by successive depolarizing test pulses. Recordings were obtained by step depolarizations lasting for 45 ms from -70 mV in 10 mV increments to between -50 mV and +30 mV.

Figure 3A shows the voltage-dependent currents induced from a DRG neuron when the cell membrane potential was changed by a ramp protocol from +90 mV to -190 mV, for 900 ms in Ringer's solution containing 5, 10, 20, and 45 mmol/L $K^+$. The currents were clearly nonlinear, showing inward rectification. They increased in size in a concentra-

tion-dependent manner when $[K^+]_o$ was raised. Furthermore, the reversal potential was shifted as predicted by the Nernst equation (Figure 3B). These results strongly suggest that $K^+$ was the dominant current carrier.
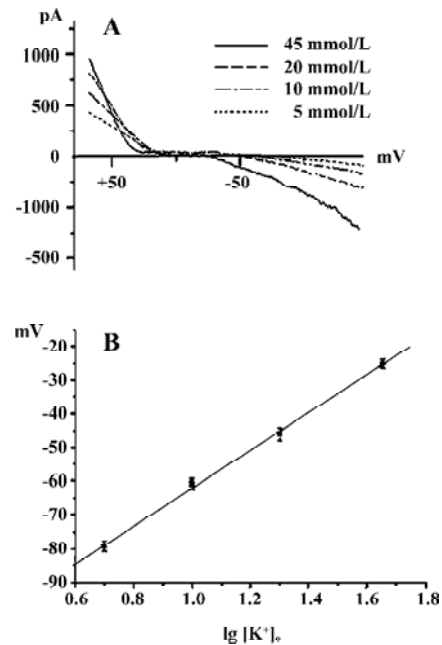


**Figure 3.** Inwardly rectifying $K^+$ currents in DRG neurons. (A) *I–V* curves of the whole-cell currents recorded from a single DRG neuron in different $[K^+]_o$ (5, 10, 20, and 45 mmol/L)-containing Ringer's solution. The cell membrane potential was ramped from +90 mV to -190 mV for 900 ms; (B) Average reversal potential of the currents determined for 6 cells was plotted against lg $[K^+]_O$. The reversal potential changed with the increase in $[K^+]_O$ (■) as predicted by the Nernst equation (solid line).

$Ba^{2+}$ can block Kir channel currents in a dose-dependent manner. Figure 4A shows the effects of 300 µmol/L $Ba^{2+}$ on the inwardly rectifying currents in a DRG neuron induced by a ramp voltage change from 0 mV to -90 mV in 45 mmol/L $K^+$-containing Ringer's solution. $Ba^{2+}$ 300 µmol/L significantly reduced the basal inward currents at 80 mV by 23%±10% (*n*=6; Figure 4B). This result implies that the inward currents mediated by inwardly rectifying $K^+$ channels existed.

$GABA_B$ receptors have been reported to be highly expressed in DRG neurons[14]. To test whether the inwardly rectifying currents in the DRG were regulated by the G protein-coupled receptor, we used the $GABA_B$ receptor agonist baclofen to activate the receptor in DRG neurons. Figure 5A, 5B show that the inward currents were markedly increased in magnitude with time following the perfusion of 100 µmol/L baclofen. An increase of 260%±140% (*n*=6) in
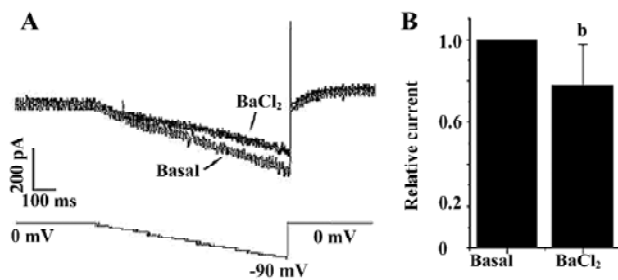
**Figure 4.** Effects of $BaCl_2$ on inwardly rectifying $K^+$ currents of rat DRG neurons. (A) Currents were obtained by a voltage ramp between 0 mV and -90 mV from a holding potential of 0 mV in 45 mmol/L $K^+$-containing Ringer's solution; (B) Statistical analysis (*t*-test) of the effects of $BaCl_2$ (300 µmol/L) on the inward currents at -80 mV. *n*=6. Mean±SD. [b]$P<0.05$ *vs* Basal. $t=2.823$, $>t(5)=2.571$.

the current obtained at -80 mV was found with 100 µmol/L baclofen (Figure 5C). The currents enhanced by baclofen were considerably suppressed by addition of 300 µmol/L $Ba^{2+}$ (Figure 5A, 5B). The currents obtained in the presence of 300 µmol/L $Ba^{2+}$ were actually smaller in magnitude than the basal currents (Figure 5A–5C), which may be due to the fact that $Ba^{2+}$ also suppressed the intrinsic fraction currents mediated by the Kir channels.

To further confirm that the activation of G protein can activate the inwardly rectifying currents of the DRG neurons, changes in inward currents were studied when guanosine triphosphate (GTP) in the pipette solution was omitted and replaced with 300 µmol/L $GTP_\gamma S$, a nonhydrolysable GTP analogue which can activate the G protein[18]. Figure 6A shows

the inward currents markedly increased in magnitude with time following the inclusion of 300 µmol/L $GTP_\gamma S$. An increase of 136%±38% (*n*=6) in the current obtained at -80 mV was found with 300 µmol/L $GTP_\gamma S$ included in the pipette (Figure 6B). The currents enhanced by $GTP_\gamma S$ were considerably suppressed by the addition of 300 µmol/L $Ba^{2+}$ (Figure 6A, 6B). These data demonstrated that the GIRK channels expressed in DRG neurons contributed to the inward currents activated by G proteins.

## Discussion

Besides the heart and pancreas, GIRK distributes in the nervous system. Earlier studies have demonstrated GIRK1–4 mRNA distribute distinctly in rat brains[19–21]. In adult mice, GIRK2-like immunostaining was detected in lamina II in all levels of the spinal cord, but not in the DRG[22], suggesting that functional GIRK channels of adult mouse DRG neurons were composed of other GIRK subunits except GIRK2. Furthermore, Kanjhan *et al* reported that no functional GIRK currents were recorded in P0 mouse DRG neurons[23], and they hypothesized that the functional expression of GIRK channels might increase with exposure to sensory of painful stimuli during postnatal development. In rats, it has been shown that GIRK1–3 mRNA are localized in DRG neurons at embryonic d 17[24]. In the present study, we demonstrated the transcription of GIRK1–4 in adult rat DRG neurons. Our results indicate that GIRK1–3 mRNA may continuously transcript from embryonic day to adult time in rat DRG neurons, suggesting that the 4 subunits are all candidates for func-
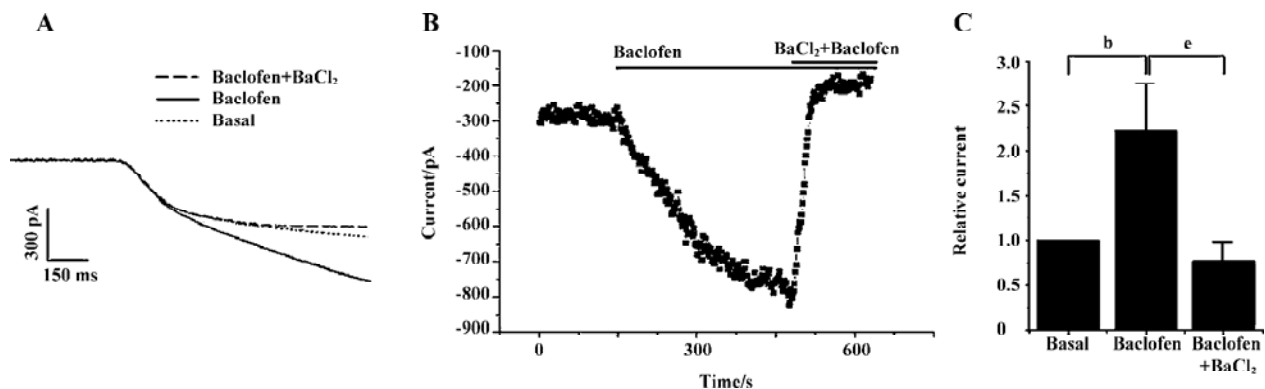


**Figure 5.** Baclofen increases inwardly rectifying $K^+$ currents of rat DRG neurons. (A) Current curves were obtained by a voltage ramp between -40 mV and -100 mV from a holding potential of -40 mV in 45 mmol/L $K^+$-containing Ringer's solution. Baclofen (100 µmol/L) significantly increased the inward currents. Addition of $BaCl_2$ (300 µmol/L) markedly suppressed the currents to an even smaller magnitude than those recorded before baclofen was added (basal currents). (B) *I–T* curve of baclofen (100 µmol/L) effect on inwardly rectifying $K^+$ currents was obtained at -80 mV in 45 mmol/L $K^+$-containing Ringer's solution. (C) Statistical analysis (*t*-test) of the effects of baclofen (100 µmol/L) or baclofen and $BaCl_2$ (300 µmol/L) on the inward currents at -80 mV. *n*=6. Mean±SD. [b]$P<0.05$ *vs* Basal. $t=49.135$, $>t(5)=2.571$. [e]$P<0.05$ *vs* Baclofen. $t=52.221$, $>t(5)=2.571$.
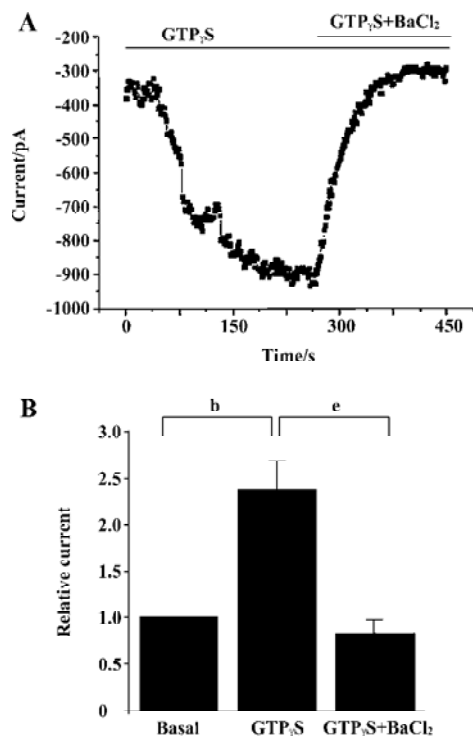
**Figure 6.** GTP$_\gamma$S increases inwardly rectifying K$^+$ currents of rat DRG neurons. (A) *I–T* curve of GTP$_\gamma$S (300 µmol/L) or GTP$_\gamma$S and BaCl$_2$ (300 µmol/L) effect on inwardly rectifying K$^+$ currents was obtained at -80 mV in 45 mmol/L K$^+$-containing Ringer's solution. GTP$_\gamma$S (300 µmol/L) significantly increased the inward currents as they diffused into the cell body. The addition of BaCl$_2$ (300 µmol/L) markedly suppressed the currents to even smaller in magnitude than initial currents (basal currents). (B) Statistical analysis (*t*-test) of the effects of GTP$_\gamma$S (300 µmol/L) or GTP$_\gamma$S and BaCl$_2$ (300 µmol/L) on the inward currents at -80 mV. *n*=6. Mean±SD. $^b$*P*<0.05 *vs* Basal. *t*=49.332, >*t*(5)=2.571. $^e$*P*<0.05 *vs* GTP$_\gamma$S. *t*=52.431, >*t*(5)=2.571.

tional GIRK channels in the DRG neurons of adult rats, and also indicating that the subunit compositions of GIRK channels may be changed with different developmental stages in different species.

GIRK channels are widely regarded as postsynaptic effectors in the CNS, primarily because of the clear dissociation of the postsynaptic and presynaptic inhibitory effects of multiple neurotransmitters in hippocampal pyramidal neurons[25]. GIRK1 and GIRK2 were also found almost exclusively in postsynaptic membranes of putative excitatory synapses in the outer layer of the substantia gelatinosa of the dorsal horn[26]. The activation of postsynaptic GIRK1 and/or GIRK2-containing channels in the spinal cord dorsal horn represents a powerful means by which intrathecal µ- and δ-selective opioid agonists evoke analgesia[26]. In the present study, GIRK channels are found functionally expressed in DRG neurons, producing further molecular bases in the regu-

lation of neuronal excitation which modulates sensory information conveying from the periphery to the spinal cord.

GABA can depolarize A- and C-type cells of DRG and decrease input resistance, mainly due to the activation of GABA$_A$ receptors and anionic ionotropic receptors expressed in rat DRG neurons[27]. The GABA$_B$ receptor has also been reported to be present in DRG neurons and play important physiological roles[14,28,29], and to modulate N-type calcium channels through G(o) pathways[14,28–30]. GABA inhibits peptide secretion through the activation of GABA$_B$ receptors that are functionally coupled to pertussis toxin-sensitive G proteins in peripheral sensory neurons from embryonic chick DRG[31]. In the present study, we found the presence of GABA$_B$ receptor-GIRK channel complexes on DRG neurons. These findings provide possible mechanism underlying presynaptic inhibition through the activation of GABA$_B$ receptors in peripheral sensory neurons.

## Conclusion

The mRNA for GIRK1–4 were detected in the DRG of adult rats. GTP$_\gamma$S enhanced inwardly rectifying K$^+$ currents of the DRG neurons, while Ba$^{2+}$ inhibited such currents. The GIRK channels were shown to couple to GABA$_B$ receptors as baclofen increased the inwardly rectifying K$^+$ currents. These results demonstrate that the expression of GIRK channels and the presence of GABA$_B$ receptor-GIRK channel complexes in DRG neurons of adult rats.

## Acknowledgements

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